Pesticides detection by a miniature microbial fuel cell under controlled operational disturbances

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Abstract

The Microbial Fuel Cell (MFC) technology holds enormous potential for inexpensive real-time and onsite testing of water sources. With the intent of defining optimal operational conditions, we investigated the effect of environmental factors (changes in temperature, pH and ionic strength), on the performance of a single chamber miniature MFC sensor. The pH of the influent had the greatest effect on the MFC performance, with a 0.531 ± 0.064 μA cm^{-2} current variation per unit change of pH. Within the range tested, temperature and ionic strength had only a minor impact (0.010 ± 0.001 μA $°C^{-1}$ cm^{-2} and of 0.027 ± 0.003 μA mS^{-1} cm cm^{-2} respectively). Under controlled operational conditions, for the first time, we demonstrated the ability of this biosensor to detect one of the most commonly applied pesticides worldwide, atrazine. The sensitivity to atrazine was 1.39 ± 0.26 ppm^{-1} cm^{-2}, with a detection range of 0.05 – 0.3 ppm. Guidelines for systematic studies of MFC-biosensors for practical applications through a factorial design approach are also provided. Consequently, our work not only enforces the promise of miniature MFC-biosensors for organic pollutants detection in waters, but it also provides important directions towards future investigations for infield applications.

Keywords – Atrazine; Biosensors; Factorial design; Formaldehyde; Microbial Fuel Cell; Water;

Introduction

Water lies at the crux of sustainable development. It is essential for poverty alleviation, public health, food and energy security, and ecosystem quality. Yet, much of the world's population faces serious freshwater challenges. 663 million people are currently without access to safe drinking water, 2.4 billion lack access to adequate sanitation and almost half of the world population will live in areas of high water stress by 2030 (WHO). Moreover, the available water sources can be contaminated by a multitude of compounds (heavy metals, pesticides, pharmaceuticals). The UN has defined the provision of clean water and adequate sanitation for all as one of their 17 Sustainable Development Goals by 2030. To achieve this goal, effective water management is critical and implies the deployment of low-cost, real time and onsite monitoring systems for water quality.

Microbial fuel cells (MFC) have shown promising potential for water quality monitoring. In an MFC, electrogenic microorganisms are utilised to degrade organic matter and generate electricity. When the electrogenic biofilm is subjected to a bioactive compound, a change in the current generated is observed, which, within a specific range, will depend on the compound concentration (Chouler and Di Lorenzo, 2015; Yang et al., 2019). As such, MFCs can be used for the quantitative and qualitative assessment of water quality (Kim et al., 2007). The interest on MFC sensors lies on the simplicity of operation (i.e. no need for an external transducer), as well as rapid response times (Di Lorenzo et al., 2014), robust long-term operation (Kim et al., 2003), self-sustainability (Yu et al., 2017), low cost (Chouler et al., 2017) and ability to respond to a wide range of toxic compounds (Abrevaya et al., 2015; Yang et al., 2016a).

MFC-biosensors have been developed for the detection of heavy metals, such as Pb^{2+} and Hg^{2+} (Kim et al., 2007; Yu et al., 2017), Ni (Stein et al., 2012), Cr^{6+} (Wang et al., 2016), Cd^{2+} (Di Lorenzo et al., 2014; Yu et al., 2017), Cu^{2+} (Jiang et al., 2015; Shen et al., 2013), as well as for

the monitoring of formaldehyde (Yang et al., 2016a, 2016b) and the biochemical oxygen demand (BOD) (Di Lorenzo et al., 2009; Kim et al., 2003; Peixoto et al., 2011) in water. So far, there are only two reported cases focused on trace organic compounds, these being diazinon (an organophosphate insecticide) (Kim et al., 2007) and bentazon (a herbicide) (Stein et al., 2012). A deeper understanding of the dose-current response relationship of such compounds is, however, needed. Moreover, many MFC biosensing studies rely on the use of macro-scale two chamber systems (Jiang et al., 2015; Kim et al., 2007; Stein et al., 2012), which exhibit additional operating costs due to the control of the catholyte, and increased capital cost of design. Therefore, the use of single chamber systems, coupled with the concept of miniaturisation (Qian and Morse, 2011), is particularly attractive. Such devices pave the way towards simplified, fast-response, cost-effective biosensing devices (Chouler et al., 2017) with improved analysis times, reliability and sensitivity due to enhanced mass transfer processes within the cell (Di Lorenzo et al., 2014; Yang et al., 2016a).

The response of MFCs-biosensor may be affected by changes in natural conditions, such as temperature, pH, salinity and BOD (Peixoto et al., 2011; Yang et al., 2016b). Such factors may affect the bioreceptor performance (Wang et al., 2016), and weaken the response of the MFC towards toxicants (Jiang et al., 2016). The impact of simultaneous changes in conditions may also lead to potential false warnings (Yang et al., 2016a). The effects of such factors must, therefore, be understood, and properly controlled when operating a MFC as a sensor. Some work has been conducted to this end. For instance, it was observed that low temperatures can slow down the current response (by almost 50% between 30 and 20°C) of a sediment-based MFC for monitoring of faecal contamination in groundwater (Velasquez-Orta et al., 2017). Additionally, high pH and low temperature were shown to significantly affect the treatment efficiency (by up to 55%), and thus the sensing capability towards Cr^{6+} of a two-chamber MFC (Wang et al., 2016). A rigorous study on the incidence that operational parameters have on the

performance of miniature single chamber MFC-biosensors, which is a critical step in enabling this technology for real applications, is still missing. Moreover, a strategy to fully understand the combined effect of environmental factors and multiple contaminants in water on MFC-biosensors is required (Wang et al., 2013).

In this context, this work investigates the effect of environmental conditions (temperature, pH and ionic strength) on the performance of a miniature single chamber MFC-biosensor for pesticides detection in water. A guideline for systematic future studies, aimed at determining the individual and interactive effects of environmental factors and multiple toxicants on the response of the MFC biosensor, is also provided. Finally, the miniature MFC is tested as a sensor for the detection of toxic compounds in water. Firstly, formaldehyde is used as a model toxicant, to allow comparisons with other MFC-biosensor studies. Afterwards, the capability of the miniature single chamber MFC to detect atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,4-triazine) is investigated.

Experimental

Materials

All reagents used were of analytical grade and purchased from Sigma-Aldrich and Alfa Aesar. All solutions used were prepared with reverse osmosis purified water. Artificial Wastewater (AW) was used as the feedstock and prepared as previously described (Chouler et al., 2017). Potassium acetate was added to AW at 100 mM. The medium was autoclaved prior to use.

Microbial fuel cells design and operation

A single chamber membrane-less miniature MFC was manufactured in polydimethylsiloxane (PDMS) with the replica moulding technique, starting from a 3D printed master structure. The MFC has total anodic chamber volume of 128 μL (length = 8 mm, width = 4 mm, height = 4 mm). The exposed surface area of the anode and cathode (both made of untreated carbon cloth, type-B, E-Tek, USA) was 0.32 cm^2 each, and the cathode was open to air.

All MFCs were fed with AW and connected to a multi-channel peristaltic pump (Ecoline, Ismatech, Germany) *via* Pharmed® BPT tubing, ID 1.6 mm (Cole-Parmer, UK). The anode and cathode were connected to a voltmeter (ADC-24 Pico data logger, Pico Technology, UK) and to an external load to polarise the cell and monitor the cell potential under closed circuit conditions. The experimental rig shown in Figure 1. The operating temperature was controlled by placing the MFCs inside an incubator. Enrichment of the electrochemically active bacteria at the anode was performed over a period of seven days. MFCs were fed under continuous recirculating conditions with AW containing 1% *v/v* mixed culture of bacteria (anaerobic sludge provided by Wessex Water, wastewater treatment in Avonmouth, UK), which was replaced daily. MFCs were first operated under open circuit conditions for up to 2 h and then connected to an external load of 1 kΩ. After enrichment, the MFCs were continuously fed with AW containing no bacteria. Polarisation experiments and analysis were performed as

previously described (Chouler et al., 2017). After polarisation, the MFCs were operated at the external resistance that gave the optimal power performance.

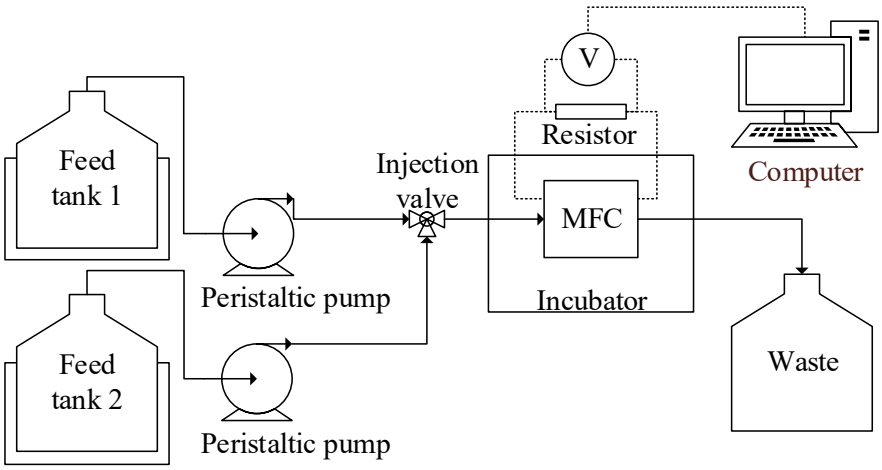

Figure 1: Experimental set up. Disturbances (e.g. change in pH, ionic strength, labile organic content or a toxic compound) are introduced *via* a three-way valve prior to the MFC using an alternative feed tank (1 & 2). Feed tank temperature is controlled by a water bath.

Testing the MFCs as biosensor

To perturb the operating conditions of the inlet solution (i.e. changes in labile organic carbon content, pH, ionic strength and introduction of a toxicant), a three-way valve prior to the MFC was used (Figure 1). All tests were carried out in triplicate. When the MFC was tested as sensor for the labile organic carbon content in water, the concentration of potassium acetate in AW was varied between 0.1 – 200 mM. For all other tests, the concentration of potassium acetate was maintained at 100 mM.

To determine the impact of temperature on the MFC, the temperature of AW was set to a range of values between 10 – 40 °C by means of an incubator for the MFC and of a water bath for

the feed tank. To investigate the effect of pH, the MFCs were fed with AW at pH values between 6.3 and 12.5. To determine the effect of ionic strength, NaCl was added to AW at concentrations between 0 and 1.8 M (corresponding to conductivities between 9.7 – 111.9 mS cm^{-1}). These concentrations were set to mimic freshwater (0 M), brackish water (0.05 – 0.3 M), seawater (0.6 M) and hyper-saline lakes (1.8 M) (Miyahara et al., 2015). Conductivity of each solution was determined using a conductivity probe (CON 110 Series, Oakton, US). Solutions were fed to the MFCs until a steady-state was established, which was defined as the point where the change in potential over time, $\delta mV/_{\delta t}$, was ≤ 0.02 mV min^{-1}.

Subsequently, the MFCs were tested for pesticide detection. Formaldehyde (10 - 2000 ppm) and atrazine (0.05 – 10 ppm) were individually tested as model toxicants. The tests involved feeding the MFCs with AW containing 100 mM of potassium acetate and the specific toxicant for the specified time, under controlled conditions of pH, temperature and ionic strength. After being exposed to the target compound, the MFCs were fed with fresh AW containing 100 mM potassium acetate and no toxicant. To avoid irreversible damage to the anodic biofilm, only one test was performed per day per fuel cell.

Calculations

The sensitivity of the MFC towards a specific disturbance applied to the system was calculated as:

$$sensitivity = \frac{\Delta I}{\Delta d \times A} \qquad \text{Equation 1}$$

Where ΔI (μA) is the unit change in the current output, Δd is the unit change in the disturbance (acetate concentration mM, formaldehyde % *v/v*, atrazine ppm) and A = 0.32 cm^2 is the anodic macro surface area.

For toxicant tests, the current variability over time was offset by normalising the current at time t, I_t, by the baseline current, I_B, to determine the normalised current, I_N, as:.

$$I_N = \frac{I_t}{I_B} \qquad \text{Equation 2}$$

The sensitivity of the normalised current response from the MFC was then referred against the anode macro surface area to give the toxicant sensitivity, and calculated as:

$$toxicant\ sensitivity = \frac{\Delta I_N}{\Delta d \times A} \qquad \text{Equation 3}$$

Where ΔI_N (-) is the unit change in the normalised current output. The ratios $\Delta I/\Delta d$ and $\Delta I_N/\Delta d$ were obtained from the linear slope of the respective current response *versus* disturbance magnitude curve. When analysing a current *versus* time response upon application of a specific disturbance: the delay time, t_d, was defined as the time between the introduction of a disturbance and the first response from the MFC; the response time, t_{res}, was the time taken to reach 95% of the new steady-state current; the recovery time, t_{rec}, was the time for the current to reach 95% of its steady-state value after a toxic event (i.e. from when fresh AW is introduced

to the MFC). The initial rate of the current response, $r_{initial}$, was defined by the initial slope of the current *versus* time curve immediately after a toxic event.

Results and discussion

Effect of operational disturbances

To be used as a sensor for the detection of toxicants, MFCs must generate a stable current baseline. Any factor that affect this baseline is a disturbance that must be understood and eventually controlled when processing the sensor readings. In this way, current changes unrelated to the presence of a bioactive compound could be filtered out and the risk of false alarms prevented. Temperature and pH, are known to have an effect on bacteria metabolism (Li et al., 2013), while the ionic strength of the sample influences the internal resistance in MFCs (Fan et al., 2008). As such, these three parameters, defined by the environmental conditions in which the system is operated, can influence the electrochemical performance of the anodic biofilm and the MFC-biosensor outputs. With the aim of understanding how such parameters affect the performance of our miniature MFC device, temperature, pH and ionic strength of the feed solution were altered as detailed in the experimental section.

Firstly, the MFCs were enriched for one week with anaerobic sludge to build-up an electroactive biofilm onto the anode surface. Afterwards, a polarisation experiment was performed, Figure S1. The open circuit voltage (OCV) for the MFC was 87.8 $\pm$ 5.4 mV. The MFC exhibited a high internal resistance of 18 ± 1.1 kΩ, comparable to the values of other miniature MFCs in the literature (Choi et al., 2015; Chouler et al., 2017; Qian and Morse, 2011). Both the low OCV and the high internal resistance are also a consequence of the membrane-less design (Chouler et al., 2017). The maximum power density of the device was

0.359 ± 0.022 mW m^{-2} at a current density of 19.4 ± 1.2 mA m^{-2}, when operating at an external load of 30 kΩ. This external resistance was used for all subsequent tests.

When the temperature was changed, a linear current response was observed, within the range 15 – 30 °C, with a gradient of 0.010 ± 0.001 μA $°C^{-1}$ cm^{-2} ($R^2 = 0.93$), as shown in Figure A and Figure S2A. Within the range 15 – 35 °C, the total current variation was only of 8%, with a peak performance at 30 °C. Outside this range, the current output decays, probably because of inhibitory effects of temperature on the bacterial metabolism and, consequently, on electron generation (Li et al., 2013). After each temperature step-change, the system required 47.3 ± 12.6 min to reach a steady current output.

The influence on pH was much more marked (Figure 2B, Figure S2B). This result is not surprising considering the importance of pH in biochemical reactions (Yang et al., 2013). A much longer time was required to reach a steady output current upon pH changes in the feeding solution (83.8 ± 15.0 min). This slower response could be explained by considering the complex responses that MFCs have towards pH. Oxygen reduction reactions at the cathode produce an alkaline environment and bacterial metabolism at the anode generally produces weak acidic compounds (He et al., 2008). These responses may conflict or complement pH changes to the electrolyte, thus elongating the overall time required for the MFC to equilibrate to a pH change. A linear relationship between current and pH was observed within the range of 7.5 – 10.9, with a gradient (per unit change of pH) of 0.531 ± 0.064 μA cm^{-2} ($R^2 = 0.98$). Poor current outputs corresponded to low pH values. This behaviour is in agreement with previous studies and has been addressed to a reduction in microbial activity at low pH (Yang et al., 2013; Yuan et al., 2011). It is supposed that although anodic bacterial activities may be inhibited, a higher pH may favour cathodic reactions and thus improve the performance of MFCs (Yang et al., 2013). Alkaline conditions might also benefit biofilm formation in MFCs, which may lead to reduced charge transfer resistances and increased exchange current density

at the anode (Yuan et al., 2011). In light of this result, the MFC should be operated at alkaline pH values to enhance power production.

When the electrolyte conductivity was varied, the MFC current output increased up to a conductivity of 36 mS cm^{-1} (Figure 2C and Figure S2C). The time required to reach a steady-state current was much slower compared to the other two parameters tested: 127.4 ± 63.1 min. This may be explained by the gradual effects that ionic strength has on the biofilm at the anode, including changes to the physiology and growth of the microbial consortia, which might not be immediately translated into changes to the current output. Within electrolyte conductivity values of 9.7 – 18.0 mS cm^{-1} a linear correlation was observed, with a gradient of 0.027 ± 0.003 μA mS^{-1} cm cm^{-2}. The increase in current generated with the solution conductivity is associated with reduced ohmic resistances within the cell (Gu et al., 2017). Moreover, high ionic strengths are preferred by anode associated bacteria, such as *Geobacteraceae*, which has been found to grow preferentially in 0.1 M NaCl (Miyahara et al., 2015). The current decrease observed for NaCl concentrations higher than 0.3 M (corresponding to conductivities above 60 mS cm^{-1}) may be attributed to the inability of exoelectrogens to survive at high salt concentrations, with consequent reduction in the electricity generation (Gu et al., 2017; Miyahara et al., 2015).

Table S1 summarises the result obtained. As observed, within the range of values investigated, of utmost importance is to control the pH, while monitoring inlet temperature and conductivity.

While informative for progressing practical uses of MFCs as water quality sensor, understanding the individual effect of operational variables on the current generated by the MFC is unfortunately not enough (Madani et al., 2015). Real water systems contain a mixture of toxicants and organic compounds and may also exhibit simultaneous changes of operational disturbances over time. The complex and co-operative effect of such factors on the current generation needs to be known to safely and reliably interpret signals from the MFC-biosensor,

and to enable practical applications. For this to be possible, not only is important the effect of each disturbance (individual and combined) on the current baseline, but it is also key to know the response and recovery time of the system to the new conditions.

Factorial design of experiment (FDOE) has shown to be a powerful statistical method that allows the effect of several parameters and their interactions on a system response to be determined with minimised experimental effort, whilst not compromising accuracy of the results. Recently, FDOE has been used to assess MFC performance (Madani et al., 2015; Velasquez-Orta et al., 2017). These studies, however, have been limited to the analysis of up to three parameters (conductivity, temperature and external resistance (Velasquez-Orta et al., 2017), or pH and buffer concentration (Madani et al., 2015)), whereas in reality several variable factors may affect performance and sensing capability of an MFC.

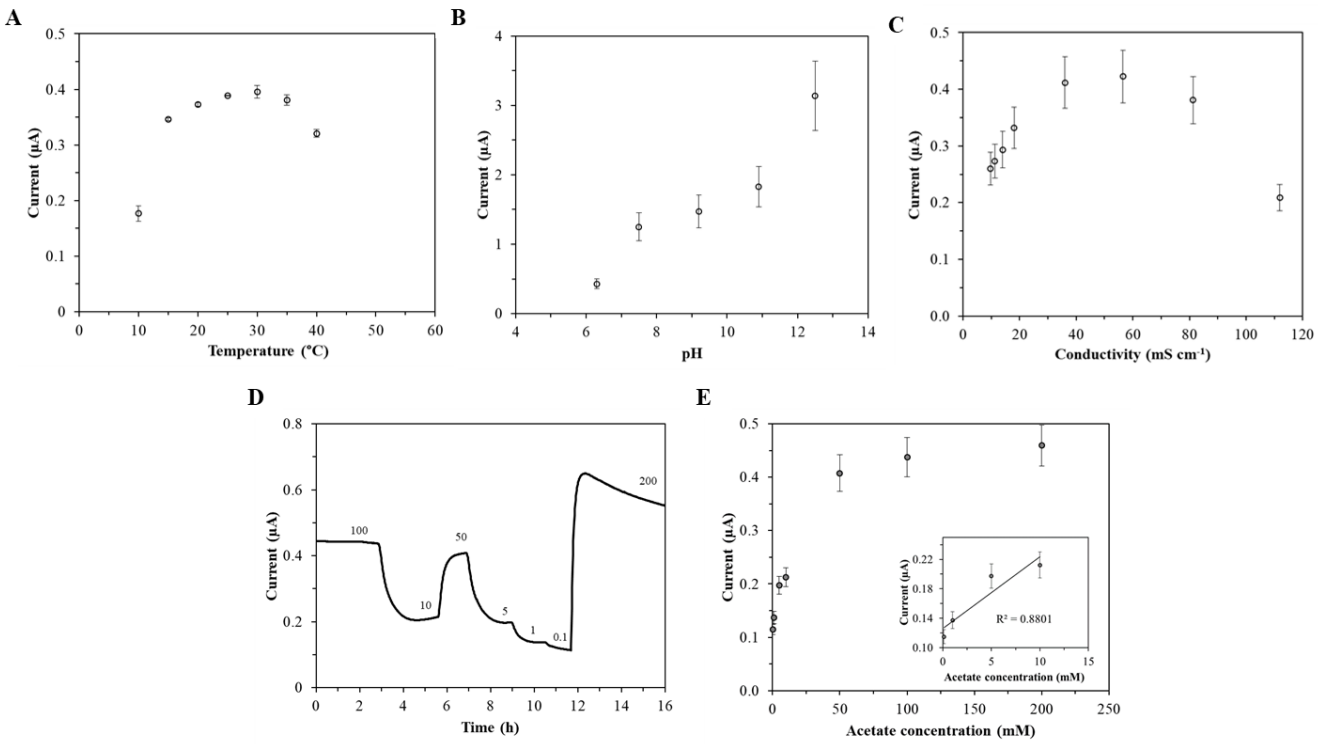

Figure 2: [A] Average steady-state current generated by the MFCs under varying temperature. Data is an average of 3 individual MFCs with up to 6% error. [B] Average steady-state current

generated under varying inlet pH. Data is an average of 3 individual MFCs with up to 12% error. [C] Average steady-state current generated under varying inlet conductivity. Data is an average of 3 individual MFCs with up to 11% error. [D] MFC response to varying inlet potassium acetate concentrations indicated (in mM) with numbers in the figure. Data is an average of 3 MFCs with 8.4% error. [E] Average steady-state current as a function of potassium acetate concentration.

For example, the external resistance has been shown to affect the recovery time and sensitivity of MFC-biosensors (Stein et al., 2012). Moreover, the shear rate (varied by flow rate though the MFC) has been shown to influence the biofilm formation and structure, thus affecting the diffusivity of toxicants into the biofilm and hence their detection by the MFC (Shen et al., 2013). Examples of operational factors are given in Figure 3. For further in-depth analysis of operational parameters and toxicant mixtures, the Supplementary Information provides a recommended methodology.

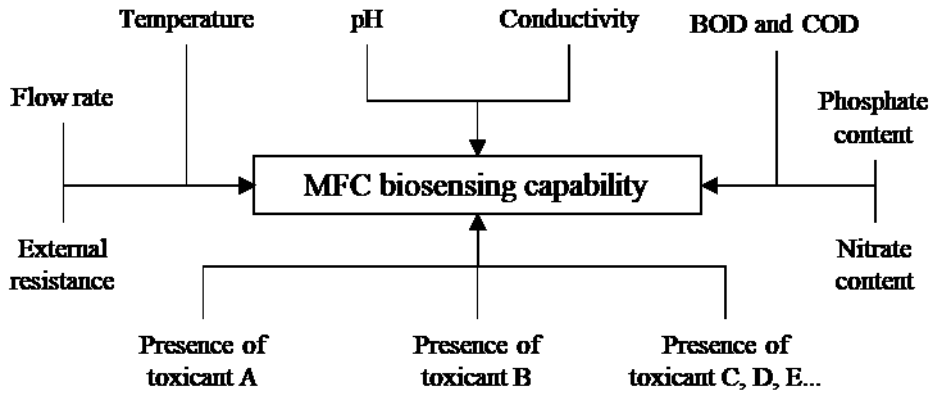

Figure 3: Example factors in waters that may affect the biosensing capability of an MFC-biosensor

For all the subsequent tests, the MFCs were operated under a controlled temperature of 20 °C and at a pH of 7.5. No NaCl was added (conductivity of 9.7 mS cm^{-1}), since the continued addition (especially at concentrations of 0.1 M and above) of NaCl has been found to alter the species present in the anodic biofilm and ultimately diminish the MFC's power performance (Miyahara et al., 2015).

The MFCs were fed with AW with varying COD values, obtained by changing the concentration of potassium acetate between 0.1 – 200 mM. The relative amperometric response is reported in Figure 2D and Figure 2E. The response to acetate follows the typical Monod model (Figure S3), revealing a Monod constant of 0.11 mM. A linear response between current output and acetate concentration was observed within the range 0.1 – 10 mM (corresponding to COD values of 10 – 1000 ppm), with a sensitivity of 0.030 ± 0.003 μA mM^{-1} cm^{-2}. The lower detection limit was 0.1 mM (10 ppm). The MFC showed a wider detection range than other labile organic content sensors reported in the literature (with detection ranges typically between 3-500 ppm of BOD (Di Lorenzo et al., 2009; Kim et al., 2003; Min and Logan, 2004; Moon et al., 2004). In this study, the wider COD range of detection may be a result of the system miniaturisation. Miniature MFCs are characterised by a higher electrode surface-area-to-volume ratio, with consequent enhancements of the mass transfer processes between the concentration of organic substrate in the bulk solution and at the surface of the electrode (Qian and Morse, 2011). The average response time of the MFCs to a change in acetate concentration was 56.8 ± 8.6 min (Table S1), which is on the same order of magnitude to similar MFCs used as BOD sensors (Di Lorenzo et al., 2009; Min and Logan, 2004). Above acetate concentrations of 100 mM, no further current enhancements were detected. As such, this concentration was considered to be saturating, in line with substrate saturation behaviour of microbial community growth kinetics (Ledezma et al., 2012), and was used for further testing of toxicants.

Testing the miniature MFC as sensor

The MFC was subsequently tested as a sensor for bioactive compounds (pesticides) in AW. Initially, formaldehyde, a commonly used disinfectant and biocide, was used as a model toxicant.

Figure 4A and Figure 4B show the effect of exposing the MFC-biosensor to formaldehyde for a period of 10 min, with results summarised in Table S2. For concentrations greater than 10 ppm, a drop in the current is observed, proportional to the concentration added, with a sensitivity of $1.43 \times 10^{-3} \pm 0.18 \times 10^{-3}$ ppm^{-1} cm^{-2}. No discernible effects were observed for formaldehyde concentrations below 10 ppm and hence data are not reported.

The MFC-biosensor showed a very fast response to the presence of formaldehyde, with a delay time, t_d, of 4.7 ± 1.8 min. For all concentrations below 2000 ppm, the current generated by the MFC returned to its original baseline current value, with an average recovery time, t_{rec}, of 67.3 ± 42.0 min. For these concentrations, it is therefore assumed that the presence of formaldehyde only caused temporary changes to the electroactive bacteria at the anode (Di Lorenzo et al., 2014). On the other hand, when a concentration of 2000 ppm was used, the baseline current was not restored. This result suggests that such levels of formaldehyde would cause permanent damage to the anodic biofilm, as previously suggested (Di Lorenzo et al., 2014). The recovery time, t_{rec}, of the current response increased as the concentration of formaldehyde increased, with a time of 28 min for 10 ppm and 117 min for 2000 ppm (Table S2). These times are significantly shorter than those reported for other formaldehyde MFC-biosensors (Table 1). Moreover, the initial rate of current response, $r_{initial}$, to formaldehyde, showed a linear response to its concentration (Table S2). Indeed, the analysis of initial rates could prove useful for rapid determination of the presence of a toxic compound in water and an indication of its

concentration, which supports the possibility to use MFCs as a rapid 'shock-sensor' for water analysis (Xu et al., 2016).

Table 1 summarises other MFC-biosensors reported that have been tested for formaldehyde detection. The MFC developed in this study demonstrates similar detection ranges reported in other studies (Dávila et al., 2011; Wang et al., 2013; Yang et al., 2016a, 2016b), and shorter t_{res} (Yang et al., 2016a, 2016b). The latter is probably a consequence of the device miniaturisation, which improves mass transfer between the bulk fluid and the biofilm at the anode (Choi et al., 2015).

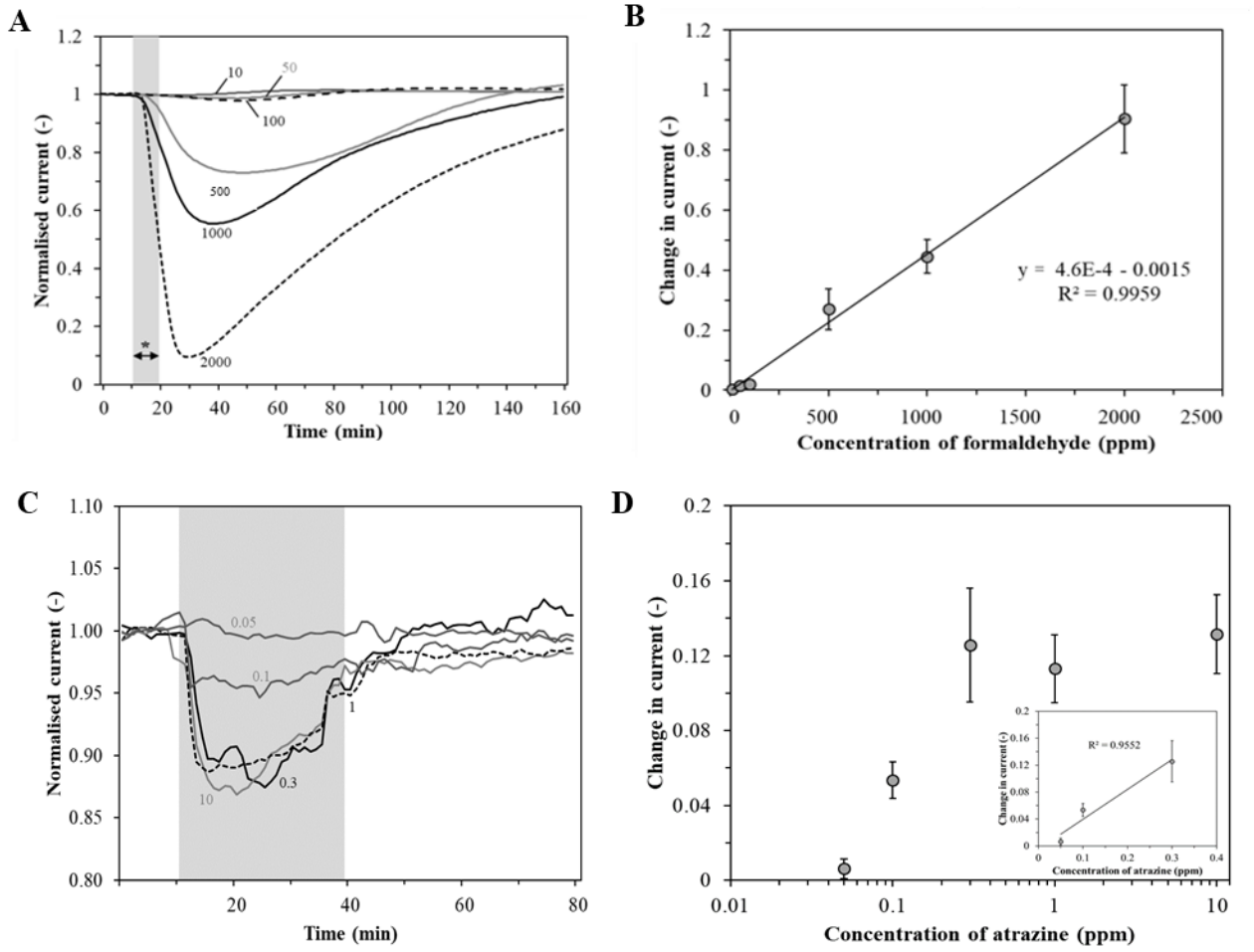

Figure 4: MFC response to pesticides. [A] Current output versus time during 10 min of formaldehyde injection, followed with AW feeding (with no formaldehyde). The numbers in the graph refer to formaldehyde concentration (ppm). The response is an average of 3 individual MFCs with up to a 12.5% error. [B] Current change after injection versus formaldehyde concentration. [C] Current output versus time during 30 min atrazine injection, followed by AW feeding with no atrazine. Number adjacent to each line indicate the concentration of atrazine (ppm). The response is an average of 3 MFCs with up to 24% error. [D] Change in current after atrazine injection versus atrazine concentration.

Subsequently, the MFC-biosensor was tested for atrazine detection. Atrazine is a member of the chlorinated *s*-triazine group of herbicides, very toxic to aquatic life and listed as a priority substance for action under the EU Water Framework Directive.

Table 1: Overview of MFC-biosensors for formaldehyde detection

Microbe assayed	**Configuration**	**Anode chamber volume**	**Formaldehyde concentration monitored (% *v/v*)**	**Response time, t_{res}**	**Recovery time, t_{rec}**	**Delay time, t_d**	**Ref.**
Microalgae wastewater consortium	Single chamber	128 µL	0.001 – 0.2	24.4 ± 7.7 min	46 ± 7.8 min	4.7 ± 1.8 min	This study
Shewanella oneidensis MR-1	Single chamber	140 µL	0.001 - 0.1	200 min	~ 175 min	N. R.	(Yang et al., 2016b)
Geobacter sulfurreducens	Two chamber	144 µL	0.1	~ 3 min	N. R	N. R	(Dávila et al., 2011)
Shewanella oneidensis MR-1	Single chamber	120 mL	0.01 - 0.1	> 9.7 h	N. R	N. R	(Wang et al., 2013)
Wild-type *Pseudomonas aeruginosa* PAO1	Single chamber, dual-channel system	90 µL	0.003 – 0.35	< 125 min	~ 330 min	N. R	(Yang et al., 2016a)

N. R.= Data not available

Figure 4C show the response of the MFC-biosensor to atrazine during 30 min of exposure. The results are also summarised in Table S2. An initial drop in the output current was observed, followed by a slow recovery towards the baseline. For concentrations between 0.05 and 0.3 ppm, the initial current drop was proportional to the concentration added, with a sensitivity of 1.39 ± 0.26 ppm^{-1} cm^{-2} (Figure 4D). Further increases in atrazine concentration did not cause marked changes in the output current. The lower detection limit for atrazine was 0.05 ppm. The average t_{rec} of the sensor was 28.6 ± 8.6 min, where greater t_{rec} were experienced on atrazine concentrations above 0.3 ppm (up to 44 min). The average t_{res} towards atrazine was 9.2 ± 3.6 min. This was the first time that the use of MFCs to detect the presence of atrazine in water is demonstrated. Whole cell biosensors for the detection of atrazine were reported, most of which adopt optical methods, utilising either microalgae (Védrine et al., 2003) or bioluminescent bacteria (Jia et al., 2012; Strachan et al., 2001). These systems demonstrate excellent detection limits, ranging from 10 fg mL^{-1} – 1 mg L^{-1} (Jia et al., 2012), 4 – 8 mg L^{-1} (Strachan et al., 2001), and 0.25 – 10 mg L^{-1} (Védrine et al., 2003). The MFC reported here has the advantages of: faster response times (detection times of atrazine microbial sensors previously reported range widely between 120 min (Strachan et al., 2001) and 180 – 300 min (Jia et al., 2012)); use of mixed anaerobic consortia rather than pure species, which simplifies practical applications; and low-cost and simple design as no external transducer is required. Since atrazine is a photosynthesis inhibitor, amperometric whole cell sensors based on cyanobacteria have also been reported (Tucci et al., 2019, Tsopela et al. 2014).

Several studies have shown atrazine biodegradation by anaerobic wastewater consortia (Ghosh and Philip, 2004), and by pure species, such as *Pseudomonas* (Behki and Khan, 1986), *Rhodococcus* (Kolekar et al., 2014), *Nicordioides* (Topp et al., 2000). The biodegradation occurs by either N-dealkylation of atrazine into deisopropylatrazine and deethylatrazine or dechlorination into hydroxyatrazine (Kolekar et al., 2014). This process is, however, very slow.

Only 45% degradation has been reported after five days residence time in an anaerobic wastewater reactor (Ghosh and Philip, 2004). Some studies have also shown the possibility to use MFCs for atrazine biodegradation. In a soil-based MFC, an 80% atrazine removal was achieved after 7 days (Domínguez-Garay et al., 2016). In a batch MFC system, an 85% decrease in atrazine concentration was observed after 24 hours. Nearly 83% of this reduction, was, however, addressed to atrazine sorption onto the biofilm or electrode surface (Werner et al., 2015). It is supposed that the mechanism of atrazine detection by the MFC sensor occurs *via* a two-step process. Firstly, atrazine is adsorbed onto the anodic surface (Werner et al., 2015). As a result, the metabolic rate of the electroactive biofilm is hindered by the addition of a mass transfer layer that limits acetate consumption and causes a drop in the output current. Subsequently, atrazine starts to be degraded by the biofilm into less complex compounds and a slow current recovery is observed. More work, however, is needed to support this hypothesis and to better understand the fate of atrazine in the MFC.

Overall, the MFC sensor showed lower sensitivity and a noisier current signal response towards atrazine with respect to formaldehyde. Indeed, formaldehyde has been proven to act as a strong biocide towards bacteria, with much stronger toxic effects than atrazine.

Conclusions

A cost-effective miniature membrane-less single-chamber MFC-biosensor for real time water quality monitoring is reported. Firstly, the effect of operational conditions (temperature, pH, ionic strength) on the sensor baseline current was systematically investigated in terms of response and recovery time and sensitivity. Within the range of values tested, the pH was found to have the most significant effect on current production, with a gradient (per unit change of pH) of 0.531 ± 0.064 μA cm^{-2}, while the ionic strength was characterised by the slowest response, which was up to 127 min.

Upon control of the operational disturbances tested, the sensing capability of the MFC device was investigated. Formaldehyde was used first as a model pesticide and atrazine was used as a case study. The MFC-biosensor demonstrated a fast response to atrazine, with a sensitivity of 1.39 ± 0.26 ppm^{-1} cm^{-2} and a lower detection limit of 0.05 ppm. This was the first time that atrazine detection by an MFC-based sensor was demonstrated. The ability of the MFC-biosensor to detect atrazine, along with fast recovery of the baseline current after exposure, shows promise for the use of this technology for cost-effective online and real time detection of this chemical. Thanks to the device miniaturisation, the MFC can be easily integrated as an inline tool (for instance with intermittent sample injection to allow long term operation at a wastewater treatment plant operation) at a wastewater treatment plant. Still, future work will necessarily need to focus on the use of real water samples to test the sensor ability to respond to the presence of toxicants in real scenarios.

Although, the effect of operational conditions on the electrochemical performance of MFCs has been previously investigated, we here report the first integrated approach that combines a study on the response of the MFC device to such factors (disturbances) with its ability to detect toxicants, to conclude with guidelines for the development of an effective factorial Design of Experiment. The latter is recommended as an invaluable tool for an in depth understanding of individual and synergistic effects of environmental conditions and multiple toxicants in real water systems. An effective Design of Experiment approach can provide a valuable and in-depth analysis of the most significant factors that affect the biosensing capability of the MFC system. Such an analysis can allow the viability of MFCs for online water quality monitoring to be fully understood and realised.

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